Lectin-Mediated Therapeutics

Rabia Hamid

Department of Biochemistry, University of Kashmir, Srinagar-India (190006)

Abstract—This review is written for general readers who would are interested in lectin biology in general and in knowing about the implications of lectin use in human health and disease, especially in therapeutics, in particular. Lectin biology along with its applications is an important facet of glycobiology and the focus today is on the many advances made in this rapidly expanding field. Drug targeting is the main focal theme of this review.
Keywords: Lectin, drug delivery, carbohydrate based vaccines

I. INTRODUCTION

The concept of lectin-mediated specific drug delivery was proposed by Woodley and Naisbett in 1988 (Bies et al., 2004). Delivery of targeted therapeutics via direct and reverse drug delivery systems (DDS) to specific sites provides numerous advantages over traditional non-targeted therapeutics (Minko, 2004; Plattner et al., 2008; Rek et al., 2009). Targeted drug delivery increases the efficacy of treatment by enhancing drug exposure to targeted sites while limiting side effects of drugs on normal and healthy tissues (Minko, 2004; Plattner et al., 2008; Rek et al., 2009). Limiting or preventing side effects in treatments is important because side effects typically lead to reduction in dosage, delay in treatment and therapy termination. Furthermore, specific drug delivery increases the uptake and internalization of therapeutics that have reduced cellular permeability (Minko, 2004; Rek et al., 2009). Direct or reverse targeting relies on identifying and utilizing unique moieties of the targeted site while protecting the active (drug) component during the delivery (Fell, 1996). In addition to specific moieties, other parameters such as the target environment and the path taken to reach the target must be considered in tailoring lectin-based DDSs (Minko, 2004; Rek et al., 2009). Drugs passing through the gastrointestinal tract are susceptible to early activation and degradation by the acidic environment and pancreatic enzymes. Alternatively, drugs administrated via the colon are vulnerable to catabolic assault by enzymes of bacterial origin (e.g. dextranase, pectinase, β-D-xylosidase, β-D-galactosidase, amylase, xylanase and β-D-glucosidase) (Guarner and Malagelada, 2003; Rek et al., 2009). However, it is possible to develop DDSs that take advantage of these bacterial enzymes. For example, a drug core in a fermentable carbohydrate coating, drug–carbohydrate conjugates (prodrugs) and drugs embedded in a biodegradable matrix are all possible designs of drugs that can utilize bacterial enzymes (Sinha and Kumria, 2001; Rek et al., 2009).

II. DRUG TARGETING

One approach to specific drug delivery as described above is prodrugs. Prodrugs are drug–carbohydrate conjugates that are delivered to the target site in an inactive form and are only activated by specific conditions at the target site. Prodrugs are typically utilized in two forms. The first type of prodrug is broken down within the target cell to form the active therapeutic or therapeutics. The second type of prodrug reacts with two or more compounds to develop the active therapeutic agent under specific intracellular conditions (Minko, 2004; Rek et al., 2009). The production of targeted DDS requires three components: the drug, a targeting moiety and a carrier. The carrier moiety binds all three components of the targeted DDS together and enhances the solubility of the entire complex (Minko, 2004; Rek et al., 2009). Targeted DDSs must meet two important conditions to be effective. First, the therapeutic agent must be protected from degradation or loss of activity, and secondly, the therapeutic agent must be released from the DDS within the target site. Therapeutic agents are typically linked to the DDS via a biodegradable spacer such as the tetrapeptide Gly-Phe-Leu-Gly, which is digested by the enzymatic activity of cathepsin B, thus liberating the therapeutic agent (Kopecek et al., 2000; Kopecek et al., 2001). Alternatively, the entire DDS may be biodegradable within the target cell (Zeisig et al., 2003). An example of this system would be the combination of horseradish peroxidase with indole-3-acetic acid. Indole-3-acetic acid is oxidized by horseradish peroxidase, thus, forming radical cations that degrade further to form cytotoxic products (Minko, 2004).

1.1. Lectin-based drug targeting

Lectin-based targeting of DDSs may be accomplished via two mechanisms (Fig. 1): direct lectin targeting and reverse lectin targeting (Plattner et al., in press). In direct lectin targeting, the DDS

has carbohydrate moieties that are recognized by endogenous cell surface lectins. In reverse lectin targeting, the DDS has exogenous lectins that recognize endogenously synthesized carbohydrate moieties on glycolipids and glycoproteins (Bies et al., 2004; Minko, 2004). Recall that human and experimental tumors display increased levels of N-linked β-1,6-GlcNAc oligosaccharides (Dennis, 1991; Fernandes et al., 1991; Dennis, 1992; Mody et al., 1995; Orntoft and Vestergaard, 1999; Couldrey and Green, 2000). This N-glycan would be an ideal moiety in reverse lectin targeting anti-cancer DDSs.
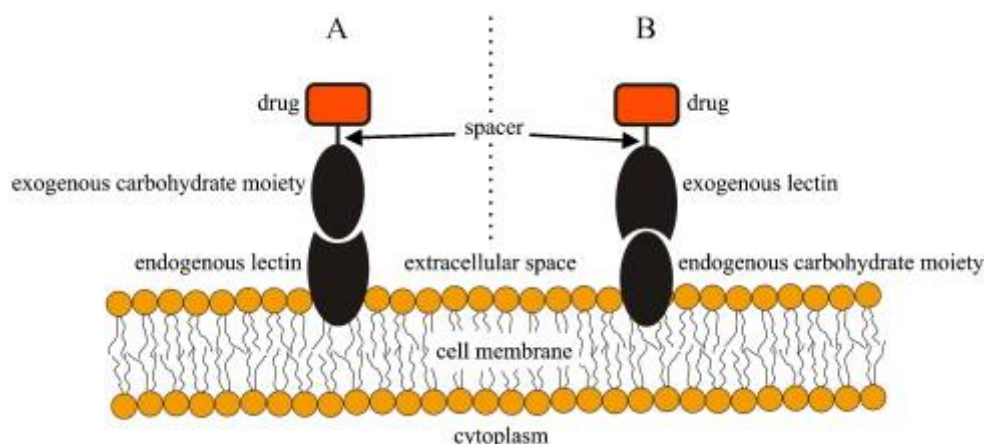

Fig. 1: Schematic illustration of direct lectin targeting (A, left) and reverse lectin targeting (B, right).
(*Illustration by Haike Ghazarian*)

Intravenous administration of anti-cancer chemotherapy reagents produces severe tissue and organ damage due to cytotoxic effects on normal cells.Lectin-based DDSs could be greatly beneficial in cancer therapy, not only due to their specific binding abilities, but also their cytotoxic and apoptosisinducing potentials (Kim et al., 1993; Gorelik, 1994; Mody et al., 1995; Ma et al., 1999; Minko, 2004; Thies et al., 2005; Plattner et al., 2008). Some lectins also have mitogenic potential. For example, lectins such as *Phaseolus vulgaris agglutinin* (PHA-L) are mitogenic to non-cancer human colon cell line CRL-1459, while cytotoxic to human colon cancer cell line CCL-220 at 1 μg/ml within 48 h of incubation (Mody et al., 1995; Gorelik et al., 2001; Sharon and Lis, 2004; Heinrich et al., 2005; Petrossian et al., 2007). Cytotoxicity of lectin-based DDSs may be exploited by two mechanisms. One mechanism would involve a non-toxic lectin conjugated to a drug which will become toxic upon activation within the target cell. The second mechanism involves using a toxic lectin that would function as a homing moiety as well as a toxic agent via apoptosis induction (Kim et al., 1993; Minko, 2004; Heinrich et al., 2005). The limitation of the second mechanism is the difficulty in identifying a lectin that would specifically be toxic towards the target cell.

Mistletoe lectin I (ML-I) is a potent antitumor cytotoxic lectin that exerts its effects by protein synthesis inhibition with high efficacy (Mody et al., 1995). Mistletoe lectin extracts, ML-I, ML-II and ML-III, have been used in European countries as experimental supplements for breast cancer therapy (Heinrich et al., 2005; Thies et al., 2005). ML-I was shown to be more toxic towards human MV3 melanoma cells *in vitro* than ML-2 or ML-3. ML-I may potentially be considered as a lectin that meets the criteria for the second mechanism since it has been used in breast cancer therapy and has been shown to be cytotoxic (Mody et al., 1995; Thies et al., 2005).

Efforts have also been made to synthesize lectin–monoclonal antibody conjugates that can specifically bind to target tumor cells and induce cytotoxic effects (Mody et al., 1995). In this system the lectin is the toxic entity and the antibody is a monoclonal tumor-specific antibody. The hope here is that virtually any tumor can be neutralized by using tumor-specific monoclonal antibodies. The toxic lectins typically used are plant lectins such as ML-I or the A-chain of ricin (Tonevitsky et al., 1991; Paprocka et al., 1992).

Plant lectins could serve as photosensitizer carrier proteins for the targeting of aberrant glycosylation on tumor cells and, with regard to antibodies, as an interesting additional weapon. Indeed, monoclonal antibodies are the most successful binding molecules in biomedicine, but their use is still limited in drug targeting, for instance by the difficulty and expensiveness of manufacturing, and the relative instability under conditions of covalent coupling with drug (Binz et al., 2005). Conversely, the structure of plant lectins confers strong resistance to heat and chemical denaturing (Liu et al., 2010; Wu et al., 2009). For instance, MorG presents a good thermodynamic stability, partially due to the absence of cysteines and consequently of disulfide bonds (Lam and Ng, 2010). The extraction of the lectin from the bark of black mulberry tree is relatively simple and the chemical linkage with PS is easy. In addition, this lectin, as others, could be probably prepared by bioengineering (Del Governatore et al., 2000). Finally, such a coupling with plant lectin appears to be

commercially realistic. With the aim to develop PS-targeting strategy, different immunoconjugates have previously been prepared between monoclonal antibodies [*i.e.*, against colorectal antigens (Hamblin et al., 2000; Savellano et al., 2005) or HER2 antigen (Serebrovskaya et al., 2009; Savellano and Hasan, 2005) and different PS molecules. Obviously, PS immunoconjugates are more selective for target cells than free PS, but can be less phototoxic on a per mole basis (Serebrovskaya et al., 2009; Price et al., 2009). On the contrary, the TrMPyP-MorG conjugate used here strongly increased the PS phototoxicity, for instance at least of 1000 times on Jurkat leukemia cells. In addition, to the best of the knowledge no conjugate has been prepared between a PS and a monoclonal antibody against T/Tn antigens. Yet, several anti-T and anti-Tn antibodies have been generated but with inconsistent results in their anti-tumor activities. A possible explanation is that previously prepared monoclonal antibodies recognize a conformational epitope constituted both by a peptide and an altered glycan, rather than the sole oligosaccharide moiety (Brooks et al., 2010). Consequently, these monoclonal antibodies can, at least partly, cross react with the target peptide on normal cells having a normal glycosylation. In contrast, some plant lectins, such as MorG have a high affinity for specific carbohydrate structure, independently of the peptide that carries the carbohydrate moiety structures. As altered glycans are over-expressed on tumor cells, plant lectins represent a powerful tool to discriminate between normal and cancer cells, by the mean of their cell surface binding to certain glycotopes, *e.g.* clusters of T/Tn antigens. This suggests that a plant lectin could be used as an efficient carrier for PS targeting, specifically into tumor cells expressing alterations of glycosylation, actually O-glycosylation. As other lectins, MorG recognizes different sets of glycotopes (high affinity to T and Tn structures), but can discriminate between subtle glycosylation modifications (Singh et al., 2007), for instance alterations of cell surface glycoconjugates between leukemic and healthy T lymphocytes.

These observations demonstrated for the first time that coupling a porphyrin with a plant lectin allows the selective elimination of leukemic cells mixed with healthy T cells, while fully preserving the functionality of normal T cells. Using different hematopoietic cell lines as well as primary lymphoid leukemia cells, the extent of cell death induced by treatment correlated with the level of MorG binding on target cells. Consequently, a useful tool to estimate the tumor sensitivity to conjugate-mediated cytotoxicity might be the determination of the level of lectin binding at 4°C on the target cell surface. However, in spite of a FITC-MorG binding lower than in sensitive leukemic cell lines, some fresh lymphoid leukemia cells were sensitive to the conjugate-mediated phototoxicity, quite similarly to the sensitive established cell lines. In addition, because cell lines with T lymphoid, B lymphoid or myeloid phenotype were similarly killed, the data suggested that the susceptibility of leukemia cells to conjugate-mediated toxicity is independent of the lymphoid or myeloid phenotypes. The degree of sensitivity of tumor cells might be related to the biochemical intracellular characteristics (such as intrinsic resistance mechanisms to cell death) rather than to the hematopoietic phenotype of leukemia cells. The results obtained with fresh leukemia samples suggested that, as compared to ALL cells, a higher binding of the lectin is required for killing CLL cells by the MorG conjugate Because the increase of ROS concentration is an essential step for PCT-induced cell death using porphyrins (Mazor et al., 2008), one possible explanation might be that the status of oxidative stress and/or antioxidant enzymes of ALL and CLL cells is different. ALL samples are characterized by a decrease of anti-oxidants (for instance, a decrease of catalase and superoxide dismutase activities (Battisti et al., 2008), and a sustained oxidative stress (due to a low NAD(P)H:quinone oxidoreductase 1 activity, known to attenuate oxidative stress (Shanafelt et al., 2005), and/or aggressive chemotherapy protocols (Battisti et al., 2008). CLL are long-lived cells *in vivo*, developing clonal resistance to apoptosis through distinct mechanisms, such as overexpression of anti-apoptotic Bcl-2 family members (Shanafelt et al., 2005). CLL cells also appear to have a complex susceptibility to oxidative stress, being able to express and release catalase extracellularly.

1.2. Carbohydrate-based vaccines and anti-adhesion therapeutics

Carbohydrate-based therapeutics is by no means a modern biomedical concept or application. Honey, for example, has been utilized for thousands of years as traditional medicine to treat microbial infections and, more recently, gastrointestinal disorders, the common cold, burns, skin ulcers, cataracts, infected wounds and asthma (Lee et al., 2008; Ferreira et al., 2009; Pourahmad and Sobhanian, 2009). Honey is a complex mixture of roughly 200 substances that include carbohydrates, proteins, organic acids, minerals, phenolic acids, enzymes, vitamins, flavonoids and other phytochemicals (Ferreira et al., 2009). Honey is a potent antimicrobial substance with antagonistic activity against pathogenic organisms such as *E. coli*, *Staphylococcus aureus* and *P. aeruginosa*. Manuka honey, for example, has been reported to have antimicrobial activity against multi-drug resistant pathogenic bacteria such as *S. aureus* and *Helicobacter pylori* (Lee et al., 2008). The antimicrobial activity of honey, in addition to other active substances, has been associated with the presence of hydrogen peroxide and minerals such as iron and copper that may contribute to the formation of highly reactive hydroxyl groups (Lee et al., 2008; Ferreira et al., 2009). However, complex carbohydrates found in honey may also contribute to the antimicrobial activity of honey. Complex carbohydrates such as the trisaccharide D(+)melezitose (α-D-glucopyranosyl-β-1,3-fructofuranosyl-α-

glucopyranoside), found in honey in concentrations of up to 3.4 mg/g, were shown to be potent inhibitors of yeast-Con A bead-binding system at low concentrations (Aso et al., 1960; Zem et al., 2006; de la Fuente et al., 2007).

1.2.1. Anti-adhesion therapy

As discussed in section "Carbohydrates in host–pathogen interactions", many human pathogens utilize cell surface glycans as either receptors or ligands to initiate adhesion and infection (Kyogashima et al., 1989; Sharon and Lis, 1989; Sharon and Lis, 2003; Thankavel et al., 1999; Zem et al., 2006; Hyun et al., 2007; Oppenheimer et al., 2008; Mukhopadhyay et al., 2009; Rek et al., 2009). Therefore, using specific carbohydrates or their analogs to interfere with the pathogen lectin–host carbohydrate interactions may prevent and treat microbial infections or diseases (Fig. 2). This is precisely the goal of anti-adhesion therapy (Zopf and Roth, 1996; Karlsson, 1998; Kelly and Younson, 2000; Sharon and Ofek, 2000; Ofek et al., 2003a; and Ofek et al., 2003b). Anti-adhesion therapy offers many advantages over conventional chemotherapies including efficacy, reduction of multiple side effects and environmental sensibility (Sharon, 2006). Many anti-adhesion carbohydrates are found as normal constituents of our diets or endogenously (Kontiokari et al., 2001; Morrow et al., 2005; Newburg et al., 2005; Sharon, 2006; Sinclair et al., 2008). Drugs using these compounds may not be safe and their safety is yet to be determined. Human milk is abundant in oligosaccharides that have inhibitory properties against surface lectins of numerous bacteria. Fucosylated oligosaccharides such as Fuc-α-2-Gal-β-4-GlcNAc are effective inhibitors of adhesion between *Campylobacter jejuni* and human cells. Infants that are breastfed with milk containing elevated levels of these oligosaccharides suffered diarrhea less frequently than those fed with milk containing low levels of these oligosaccharides (Morrow et al., 2005; Newburg et al., 2005; Sinclair et al., 2008). Sinclair et al., (2008) demonstrated the inhibition of cholera toxin binding to the GM1 receptor by sialyloligosaccharides (SOS).

Evidence from non-human cases also support carbohydrate-based anti-adhesion therapies. It was found that new born calves given lethal doses of *E. coli* K99 (F5) were cured by drinking water enriched with glycopeptides prepared from cow plasma non-immunoglobulin glycoproteins (Mouricout et al., 1990). However, difficulties with carbohydrate-based anti-adhesion therapies remain.
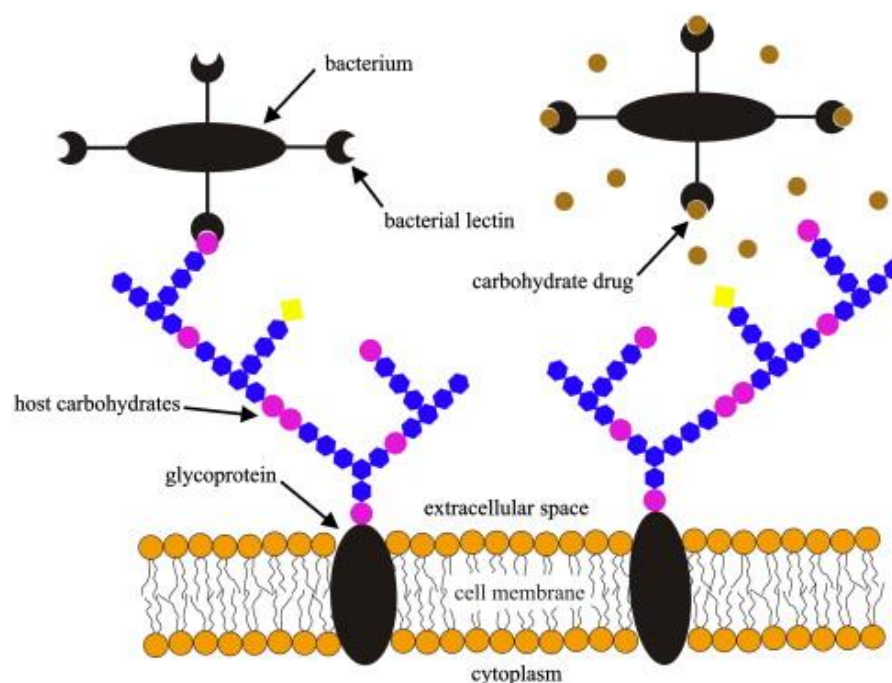

Fig.2: Schematic illustration of bacterial lectins binding to cell surface glycans of a host cell prior to infection (left) and specific carbohydrates or their analogs interfering with the bacterial lectin–host carbohydrate interactions (right). (*Illustration by Haike Ghazarian*).

Several issues that need to be addressed are: development of more potent inhibitors, expression of multiple lectins with diverse specificities by bacteria that may require multiple carbohydrates for inhibition and the low affinity of free carbohydrates for microbial lectins that may be overcome by polymeric carrier–carbohydrate conjugates (Sharon, 2006).Other agents besides carbohydrates may also be used in anti-adhesive therapies (Rek et al., 2009). Monoclonal antibodies raised against microbial cell surface carbohydrate

determinants complementary to host cell lectins will also inhibit attachment of the pathogen (Sharon, 2006). Antibodies are proteins (polypeptides) and may yet offer another anti-adhesive agent. Natural and synthetic polypeptides do exist that have demonstrated specific binding potentials (Hyun et al., 2007; Ajesh and Sreejith, 2009). Hyun et al., (2007) found that synthesized peptides had increased specificities when compared to natural lectins. The synthetic peptides, however, had binding affinities that were an order of magnitude weaker than those of natural lectins. Most naturally occurring peptides are not and cannot be used as therapeutic agents because of toxicity against mammalian cells, low tissue binding and penetrability, high cost and susceptibility to proteolytic degradation (Ajesh and Sreejith, 2009). Further investigation is required to establish a complete list of natural and synthetic peptides that may be used in anti-adhesion therapies.

1.2.2. Carbohydrate-based vaccines

Carbohydrate-based vaccine development has had a long history; dating back to the early 1920s, but it has not received much attention for the better part of the twentieth century due to efforts being focused on chemotherapeutic and antibiotic therapies (Vliegenthart, 2006; Abdel-Motal et al., 2009; Hecht et al., 2009). The steady rise in antibiotic resistance has revived interests in carbohydrate-based vaccines once again. One issue with carbohydrate-based vaccines is that polysaccharides generally induce poor immunogenic response in normal individuals and especially in high risk groups such as neonates, children two years or younger, the elderly, chronically ill individuals and immuno-compromised individuals such as HIV and chemotherapy patients (Vliegenthart, 2006; Oppenheimer et al., 2008). To overcome this weak immune response, even when specific disease carbohydrate antigens are used in vaccine development, researchers have developed multi-component vaccines (including a three component vaccine), thus strengthening the immune response (Galonic and Gin, 2007; Abdel-Motal et al., 2009; Hecht et al., 2009). The three components are: a carbohydrate antigen, an immunocarrier protein such as keyhole limpet hemocyanin (KLH) and an immunological adjuvant such as QS-21A (Vliegenthart, 2006; Galonic and Gin, 2007; Hecht et al., 2009). The carbohydrate antigen–KLH complex is processed by antigen presenting cells, and the processed antigen is then presented to T cells. This T cell activation results in a strong T cell response with cytokine release that stimulates antibody production. The immune response generated here is directed not only towards the KLH immunocarrier protein, but also towards the weakly immunogenic carbohydrate antigen (Vliegenthart, 2006; Galonic and Gin, 2007; Hecht et al., 2009). The immunological adjuvant QS-21A is non-immunogenic on its own; however, when co-administered with the carbohydrate antigen–immunocarrier protein complex, it further enhances the immune response (Kensil et al., 1991; Helling et al., 1995; Ragupathi, 1996; Hecht et al., 2009). All three components of carbohydrate-based vaccines, however, must be safe for human administration (Vliegenthart, 2006; Hecht et al., 2009).

Advances in technology make it possible to synthesize complex carbohydrates and produce designer immunogenic complexes (Vliegenthart, 2006; Galonic and Gin, 2007; Scanlan et al., 2007; Oppenheimer et al., 2008; Hecht et al., 2009). Carbohydrate vaccines that utilize these new developments offer innovative approaches to human disease mitigation (Galonic and Gin, 2007; Hecht et al., 2009).

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